# PURIFICATION OF T4 PHAGE BY ADSORPTION ON POLYLYSINE AGAROSE

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## 1. Introduction

Purification of complex viruses usually involves a series of fractionation procedures, necessarily performed under mild conditions to prevent destruction of functional structures of the virus. Density gradient centrifugation [1] and zonal ultracentrifugation [2], have been used for purification of the complex influenza virus, which has also been purified by electrophoresis in 0.16% agarose [3]. Another mild method is distribution in a two-phase system which has been used for purification of e.g. T2 phages [4]. Chromatography procedures have been employed as well: by selecting conditions for adsorption and elution of virus on calcium phosphate it is possible to remove contaminants [5]. Recently, the successful isolation of Aleutian Mink Disease virus by biospecific affinity chromatography, involving an immunoadsorption technique, has been reported [6].

Our aim has been to introduce another mild, simple, and more specific method for purification and concentration of T4 phages from the lysates of T4-infected *E. coli*. Our method involves adsorption and desorption of T4 phages from polylysine agarose. The principle is based on the observations by Katchalski that polylysine in solution interacts with viruses, bacteria, etc. [7]. The homogeneity and the biological activity of the samples obtained have been analyzed by plaque assays, gel filtration, and electron microscopy.

## 2. Materials and methods

Sepharose 2B was obtained from Pharmacia (Sweden), cyanogen bromide from Fluka (Switzerland), and poly DL-lysine-HBr (mol. wt. 15–30 000) was purchased from Sigma (USA).

The T4 phages were grown on *E. coli* strain B, in Friedlein medium [8] at pH 7.2, and the lysates of T4-infected *E. coli* were collected.

### 2.1. *Virus assay*

The concentration of T4 was calculated from plaque counts by the usual agar layer method [8].

### 2.2. *Coupling of poly DL-lysine to Sepharose 2B*

Polylysine was coupled to Sepharose 2B according to the cyanogen bromide method [9]. The conditions used were the following: 50 ml of sedimented Sepharose 2B were washed with distilled water on a glassfilter and suspended in 50 ml of cyanogen bromide solution (5 g CNBr/10 ml water) under mild stirring. After equilibration for 5 min the pH was adjusted with 2 N NaOH to pH 11.0 and kept constant for 8 min by means of a pH-stat (Radiometer pH meter 51 with Titrator 11 and autoburette unit ABU 12). After the activation reaction the gel was washed with water and mixed with 50 ml 0.3 M potassium carbonate buffer pH 9.5 containing 100 mg polylysine. The coupling was performed at room temperature for 16 hr under mild stirring, and the product was washed with 300 ml of the following solutions: 0.3 M carbonate buffer pH 9.5 containing 1 M NaCl; distilled water; 0.05 M glycine–HCl buffer, pH 3.0, containing 1 M NaCl, and finally with the carbonate buffer. A portion of the gel was washed with distilled water and acetone and dried; the amount of bound lysine was determined by amino acid analysis according to Spackman et al. [2]. The rest of the coupled polylysine was mixed with a saturated solution of glycine in carbonate buffer with pH adjusted to pH 9.5 to block remaining reactive groups.


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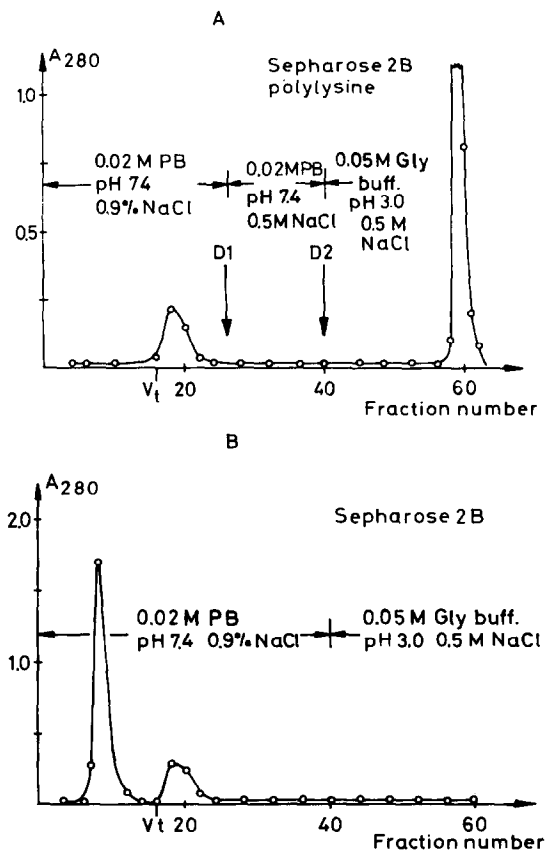


Fig. 1 A) Adsorption and desorption of T4 phages on Sepharose 2B-polylysine. For the conditions of the experiment: see the text. Adsorption and desorption was performed at pH 7.4. At D1 0.5 M NaCl was introduced in the buffer, and at D2 desorption with buffer at pH 3.0 was started; B) Gel filtration of T4 phages on Sepharose 2B. (PB = phosphate buffer, Gly buff. = glycine buffer, $V_t$ = one total column volume).

### 2.3. *Adsorption experiments*

Adsorption and gel filtration of T4 were performed at 4°C in a column on beads of polylysine–Sepharose 2B and Sepharose 2B as a control. The column dimensions were 1.4 X 19 cm, the flow rate was 4 ml/hr. The columns were equilibrated with 0.02 M phosphate buffer pH 7.4 containing 0.9% NaCl. Desorption of phages was performed with 0.05 M glycine–HCl buffer pH 3.0 containing 0.5 M NaCl. Gel filtration on Sepharose 2B was performed under the same conditions.

### 2.4. *Electron microscopic analysis*

The fractions obtained after the chromatography as well as the lysate of T4-infected *E. coli* were analyzed in the transmission electron microscope. Two percent neutralized phosphotungstic acid (PTA) or ammonium molybdate were used as contrasting agents, and the specimens were placed on carbon coated grids. Some specimens were also fixed in 2% glutaraldehyde prior to the contrasting.

The specimens were studied in a Philips 300 electron microscope, with an anti-contaminating device and an image intensifier. Through-focal series of electron micrographs were taken of each specimen.

## 3. Results and discussion

The amount of coupled polylysine was 100 mg/g dry polymer, this value being calculated from an average mol. wt. for polylysine of 20 000. After coupling of the polyamino acid the polymer was deactivated by blocking of the remaining reactive groups with glycine. This step has proven to be very important to minimize unspecific adsorption, to which coupled glycine did not contribute [11].

T4 phage has been purified in one step by adsorption and desorption on polylysine agarose. T4 phages were adsorbed on polylysine agarose at pH 7.4. No virus was eluted during adsorption and washing. The addition of 0.5 M NaCl to the washing buffer had no effect on adsorbed virus, while glycine buffer, pH 3.0 (0.5 M NaCl) eluted 96% of adsorbed virus according to ultraviolet absorption (fig. 1) and plaque assays. A control experiment with Sepharose 2B was performed under similar conditions. Fig. 1 B shows that no virus material was adsorbed on this gel, but was eluted by molecular sieving.

After repeated exposure of the coupled Sepharose to lysate from T4-infected *E. coli* a change in desorption conditions was observed. Adsorbed T4 could then be eluted with glycine buffer pH 6 containing 0.5 M NaCl, instead of requiring the more acidic buffer, pH 3. This change took place gradually and is probably due to enzymes present in the lysate. The physical properties of the gel also changed, indicating that the gel itself was affected. If, however, a partially purified virus solution was chromatographed, the adsorbent could be used several times and no change in the

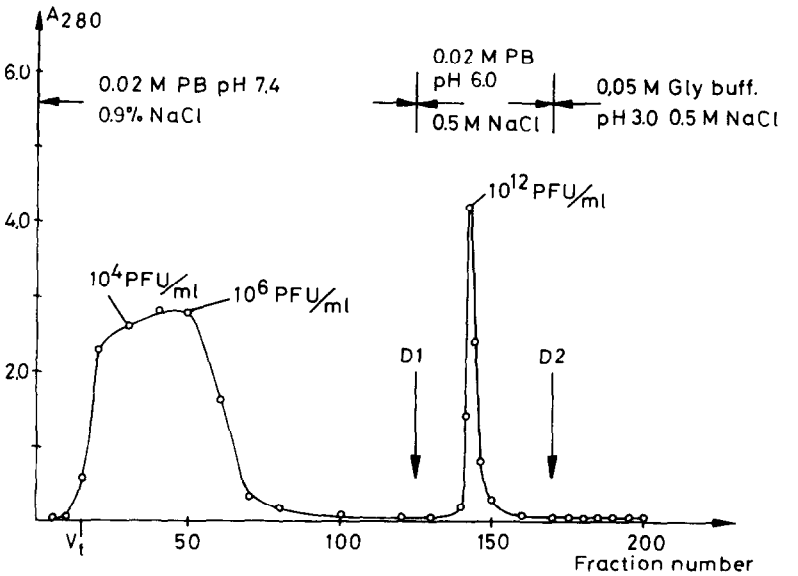


Fig. 2. Adsorption and desorption of T4 phages on Sepharose 2B-polylysine. The same column and the same conditions were used as described under fig. 1. The sample volume was 100 ml of a lysate of T4-infected *E. coli*. D1 indicates the start of desorption with 0.02 M phosphate buffer at pH 6.0 containing 0.5 M NaCl, and D2 indicates desorption with buffer at pH 3.0 containing 0.5 M NaCl.

adsorption or desorption capacities or conditions was observed nor was the gel affected.

It was shown by electron microscopy that the lysates of T4-infected *E. coli* contained several kinds of structures: cell debris (membrane fragments) often attached to T4 phages; individual T4 phages and lower molecular weight substances (plate 1). After adsorption to and elution from the column, the desorbed material: fraction no. 142–145 (fig. 2), consisted of only individual T4 phages (plate 2). Almost all bacteriophages appeared to be intact showing a projection

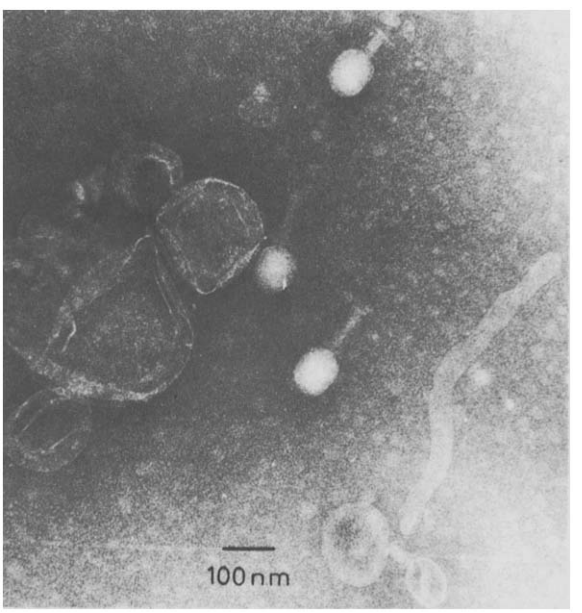


Fig. 3 a) A lysate from T4-infected *E. coli*, showing membraneous material with T4 phages.

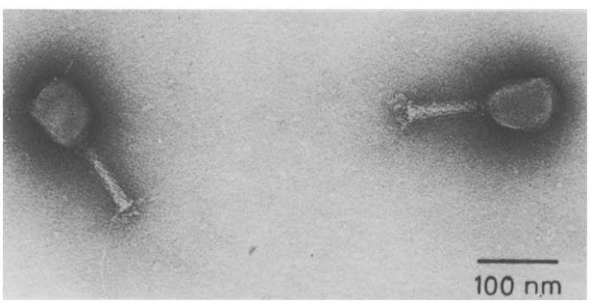


Fig. 3 b) Infectious T4 phages after desorption from polylysine agarose; the structural components of the virus seem to be intact.

of the prismatic head and a striated tail with tail fibers (plate 2).

An experiment was performed with 100 ml of a lysate of T4-infected bacteria. Fig. 3 shows an experiment with frontal analysis of virus and ultraviolet absorbing substances. The starting material contained $6 \times 10^{11}$ pfu/ml and the ultraviolet absorption at 280 nm was 5.7. Because the adsorbent was not saturated, a value of $10^{12}$ pfu adsorbed per ml adsorbent represents a minimum value of capacity. These results indicate that this adsorption technique could be of value in concentration and purification of large volumes of virus. This method employed for purification and concentration of T4 phages has resulted in a high yield of intact virus without contamination by cell debris. All other methods which we have used for fractionation of T4: differential centrifugation, density gradient centrifugation, gel filtration, partition in a two-phase system, free electrophoresis, have resulted in some contamination by cell debris and some destruction of virus particles. These observations indicate that this adsorption to polylysine residues should be an advantageous method for quantitative and mild preparation of the complex T4 phages.

Because the interaction between polylysine and the virus probably depends mainly on net charge effects, and thus is not strictly specific, this adsorption on polylysine agarose is not 'biospecific affinity chromatography', according to Porath and Kristiansen [11]. However, this modified gel seems highly selective for virus adsorption, in this case T4, from a crude bacterial lysate. Other simple or complex viruses may be adsorbed to this polylysine agarose gel, and a fractionation of these viruses could be undertaken due to different net charge on the surface of the virus.

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